

# The Environmental Estrogen Bisphenol A Inhibits Estradiol-Induced Hippocampal Synaptogenesis

Neil J. MacLusky,<sup>1</sup> Tibor Hajszan,<sup>2,3</sup> and Csaba Leranth<sup>2,4</sup>

<sup>1</sup>Center for Neural Recovery and Rehabilitation Research, Helen Hayes Hospital, New York, New York, USA; <sup>2</sup>Department of Obstetrics, Gynecology, and Reproductive Sciences, Yale University School of Medicine, New Haven, Connecticut, USA; <sup>3</sup>Laboratory of Molecular Neurobiology, Biological Research Center, Hungarian Academy of Sciences, Szeged, Hungary; <sup>4</sup>Department of Neurobiology, Yale University School of Medicine, New Haven, Connecticut, USA

Bisphenol A (BPA) is an estrogenic chemical that is widely used in the manufacture of plastics and epoxy resins. Because BPA leaches out of plastic food and drink containers, as well as the BPA-containing plastics used in dental prostheses and sealants, considerable potential exists for human exposure to this compound. In this article we show that treatment of ovariectomized rats with BPA dose-dependently inhibits the estrogen-induced formation of dendritic spine synapses on pyramidal neurons in the CA1 area of the hippocampus. Significant inhibitory effects of BPA were observed at a dose of only 40 µg/kg, below the current U.S. Environmental Protection Agency reference daily limit for human exposure. Because synaptic remodeling has been postulated to contribute to the rapid effects of estrogen on hippocampus-dependent memory, these data suggest that environmental BPA exposure may interfere with the development and expression of normal sex differences in cognitive function, via inhibition of estrogen-dependent hippocampal synapse formation. It may also exacerbate the impairment of hippocampal function observed during normal aging, as endogenous estrogen production declines. **Key words:** bisphenol A, CA1, estradiol, hippocampus, spine synapse density. *Environ Health Perspect* 113:675–679 (2005). doi:10.1289/ehp.7633 available via <http://dx.doi.org/> [Online 24 February 2005]

Natural and man-made chemicals in the environment can exert hormone mimetic or antagonist activity. Bisphenol A (BPA), a widely used chemical with mixed estrogen agonist/antagonist properties, is employed in the manufacture of plastics used in dental prostheses and sealants (Suzuki et al. 2000), in the linings of metal cans used to preserve foods (Kang et al. 2003), and in such items as baby bottles (Brede et al. 2003) and the clear plastic cages used in many research institutions to house laboratory animals (Howdeshell et al. 2003). The low affinity of BPA for the cell nuclear estrogen receptors ER- $\alpha$  and ER- $\beta$  and weak bioactivity in standard tests of estrogenicity, such as the rat uterotrophic assay (Ashby 2001), have led to the conclusion that human BPA exposure is probably insufficient to elicit significant estrogenic responses [Degen et al. 2002; European Commission (EC) Scientific Committee on Food 2002; U.S. Environmental Protection Agency (EPA) 1993]. Whether the endocrine activity of BPA is accurately reflected in such tests, however, remains controversial, because of the potential for tissue and cell-specific estrogen effects (Safe et al. 2002). Of particular concern, several reports have indicated that BPA exposure inhibits sexual differentiation of nonreproductive behaviors, including maze learning behavior (Carr et al. 2003; Farabollini et al. 2002), at doses as much as 1,000-fold lower than those required for stimulation of uterine growth (Ashby 2001). The mechanisms underlying these low-dose effects remain unknown.

Sexual differentiation of the brain is believed to involve neurotrophic effects of estrogens, mediated at least in part via activation of kinase-dependent signaling cascades (Toran-Allerand et al. 1999). Kinase-associated neuroplastic responses to estrogen are also expressed in adulthood, in the cornu ammonis (CA) pyramidal neurons of the hippocampus (Bi et al. 2001; McEwen 2002). In adult female rats (Woolley and McEwen 1992) as well as nonhuman primates (Leranth et al. 2002), estradiol induces a rapid increase in CA1 pyramidal cell dendritic spine synapse density (PSSD), a response that has been postulated to involve intermediate activation of the mitogen-activated protein (MAP) kinase cascade (Bi et al. 2001). We reasoned that if BPA inhibits sexual differentiation of the rodent brain, there might also be significant interactions between estradiol and BPA with respect to the regulation of hippocampal CA1 PSSD. Consistent with this hypothesis, in rat hippocampal organotypic cultures, regulation of NMDA receptors, which are critical components of the mechanisms responsible for estrogen regulation of CA1 dendritic spine density (Woolley and McEwen 1994), has been reported to be sensitive to nanomolar concentrations of either 17 $\beta$ -estradiol (E<sub>2</sub>) or BPA (Sato et al. 2002). Therefore, in the present study, we examined the effects of estradiol and BPA, alone and in combination, on CA1 PSSD in adult ovariectomized (OVX) rats. Our results indicate that BPA does indeed have potent effects on the regulation of CA1 PSSD. However, the data demonstrate that,

rather than inducing estrogen-like responses, BPA antagonizes the rapid inductive effects of estrogen on hippocampal PSSD.

## Materials and Methods

**Animals.** Experimental protocols were approved by the Institutional Animal Care and Use Committee of Yale University, where all studies using animals were performed. Adult female Sprague-Dawley rats (250–300 g; Charles River Laboratories, Wilmington, MA, USA) were used. The rats were ovariectomized under ketamine/xylazine/acepromazine anesthesia (3 mL/kg intramuscular injection of a cocktail containing 25 mg ketamine, 1.2 mg xylazine, and 0.03 mg acepromazine in 1 mL saline).

**Morphologic studies.** One week after ovariectomy, animals were treated with estrogen, using groups of three animals per treatment condition. In the first PSSD study, 15 rats (five groups of three rats) were injected subcutaneously with either 17 $\beta$ -E<sub>2</sub> (60 µg/kg; 12 rats) or the sesame oil vehicle (200 µL; three rats). Nine of the 12 estradiol-treated animals were treated simultaneously with increasing doses (40, 120, and 400 µg/kg) of BPA (> 99% purity; Sigma-Aldrich, St. Louis, MO, USA). In the second PSSD experiment, 12 rats were injected subcutaneously (three rats per treatment) with 17 $\alpha$ -E<sub>2</sub> (45 µg/kg), BPA (300 µg/kg), a combination of 17 $\alpha$ -E<sub>2</sub> (45 µg/kg) plus BPA (300 µg/kg), or the sesame oil vehicle (200 µL) alone. Thirty minutes after injection, animals were sacrificed under deep ether anesthesia by transcardial perfusion of heparinized saline followed by a

Address correspondence to N.J. MacLusky, Center for Neural Recovery and Rehabilitation Research, Helen Hayes Hospital, New York State Department of Health, Route 9W, West Haverstraw, NY 10593-1195 USA. Telephone: (845) 786-4810. Fax: (845) 786-4875. E-mail: macluskyn@hele nhayeshosp.org

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